REMARKS

Status of the claims

Claims 57, 68-71, 87-91, 93 and 96-102 were pending. Claims 91, 93, and 96-102 have been withdrawn from consideration, but as they contain all of the limitations of the elected composition claims, they are eligible for rejoinder upon allowance of the claims under consideration.

Claim 57 has been amended as shown above to clarify the claimed subject matter. As the new matter rejections appear to be based on the assertion that a "non-naturally occurring zinc finger protein" necessarily means a zinc finger protein that has a non-naturally occurring overall structure and/or includes non-naturally occurring amino acids, claim 57 has been amended to specify that it is only the recognition helix region of the zinc finger domains that are non-naturally occurring. As this amendment does not change the scope of the claims (and therefore does not require a new search), entry thereof after final is in order such that the claims are pending as shown above.

With regard to withdrawn claims 91, 93, and 96-102, Applicants again note that these method claims contain all the limitations of the examined compositions claims. Accordingly, as these claims should be rejoined upon indication of allowable subject matter, cancellation is inappropriate. MPEP § 714.

Objections/Rejections Withdrawn

The objections to claims 57 and 68-71 have been withdrawn. (Final Office Action, page 11). In addition, the rejection of claims 57 and 68-71 under 35 U.S.C. § 112, 2nd paragraph as allegedly unclear has also been withdrawn. (Final Office Action, page 12). Finally, the previous rejections under 35 U.S.C. § 103(a) over various co-owned patents were withdrawn pursuant to 35 U.S.C. § 103(c)(1). (Final Office Action, pages 13-14).

35 U.S.C. § 112, 1st paragraph, written description (new matter)

Claims 57 and 68-71 were again rejected under 35 U.S.C. § 112, 1st paragraph as allegedly containing new matter not described in the originally filed specification, namely

a zinc finger protein comprising at least one zinc finger domain with a non-naturally occurring recognition helix. (Office Action, pages 11-13). In addition, claims 57 and 68-71 were newly rejected under 35 U.S.C. § 112, 1st paragraph as allegedly containing new matter for reciting "non-naturally occurring." (Final Office Action, page 14-15).

These rejections appear to be based on a misunderstanding of the claimed subject matter (Final Office Action, page 12 and page 15):

> Claims 57 and 68-71 are complexes in cells in which a generic zinc finger comprising at least 3 fingers, one of which recognizes a generic non-naturally finger is bound to cellular chromatin in a region sensitive to DNAseI digestion.
>
> The specification teaches generally to mutate known domains, but does not teach the structure of all zinc finger proteins, nor does it teach what mutations are non-naturally occurring. Still further, the original claims and the original specification fail to demonstrate any possession of the genera as claimed. At best, non-naturally occurring amino acids are claimed to be an inclusive genera in the overall genera of the invention...however such fails to evince that Applicant possessed only those with non-naturally occurring amino acids, as a genera, much less the larger genera of any non-naturally occurring zinc finger protein.

However, the claims are not directed to zinc finger proteins with at least 3 fingers in which one of the fingers "recognizes a generic non-naturally finger is bound to cellular chromatin in a region sensitive to digestion with DNAseI." (Final Office Action, page 12). Nor are the claims drawn to non-naturally occurring amino acids, to unknown zinc finger proteins or to zinc finger proteins that have naturally occurring recognition helix regions. Rather, the claims are drawn to zinc finger proteins in which all the fingers are non-naturally occurring in that the 7 amino acids of the recognition helix region are designed and/or selected. For the reasons of record and reiterated herein, the **claimed** subject matter is fully described in the as-filed specification and no new matter has been added to the claims by amendment.

To reiterate, the proscription against the introduction of new matter in a patent application (35 U.S.C. §§ 132 and 251) serves to prevent an applicant from adding information that goes beyond the subject matter originally filed. *See, e.g., In re*

Rasmussen, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981) and MPEP § 2163.06. However, literal description of claimed subject matter is never required (M.P.E.P. § 2163.02):

> The subject matter of the claim need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement.

Thus, the written description requirement is satisfied if the specification reasonably conveys possession of the invention to one skilled in the art. *See, e.g., In re Lukach*, 169 USPQ 795, 796 (CCPA 1971).

Second, the disclosure must be read in light of the knowledge possessed by the skilled artisan at the time of filing, for example as established by reference to patents and publications available to the public prior to the filing date of the application. *See, e.g., In re Lange*, 209 USPQ 288 (CCPA 1981). Not only must the disclosure be read in light of the knowledge possessed by one of skill in the art, but the burden is on the Examiner to provide evidence as to why a skilled artisan would not have recognized that the applicant was in possession of claimed invention at the time of filing. *Vas Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111 (Fed. Cir. 1991); *In re Wertheim*, 191 USPQ 90 (CCPA 1976).

Likewise, definiteness of claims (including the term "non-naturally occurring") must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of the prior art and (3) claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. *See, e.g., Energizer Holdings, Inc. v. ITC,* 77 USPQ2d 1625 (Fed. Cir. 2006). In other words, the terms at issue must be read in context of the application and field of endeavor.

Applying this well-settled law to the instant case, it is clear that the specification as filed conveys that Applicants were in possession of the claimed subject matter, at the time of filing. With regard to a zinc finger protein comprising at least 3 fingers, each with a non-naturally occurring recognition helix, it is clear that a designed and/or selected zinc finger protein as described in detail and exemplified in the specification is inevitably a zinc finger protein whose fingers comprise non-naturally occurring recognition helices (page 5, lines 14-25 (paragraph [0019] pf published application); page 6, lines 20-31

(paragraph [0025] of the published application); page 17, lines 4-20 (paragraph [0070] of published application); Example 8 (paragraphs [0110], [0111] and [0114] of published application); Table 1 which shows exemplary non-naturally occurring zinc finger recognition domains; Example 15 (paragraph [0158] of published application), emphasis added):

> In another embodiment, an accessible region is identified within a region of interest and a ZFP target site is located within the accessible region. A ZFP that binds to the target site is designed. The designed ZFP can be introduced into the cell, or a nucleic acid encoding the designed ZFP can be designed and the designed nucleic acid can be introduced into the cell, where it will express the designed ZFP. Methods for the design and/or selection of ZFPs that bind specific sequences are disclosed in U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Methods for selection include, but are not limited to, phage display and in vivo selection.
>
> In methods comprising introduction of an exogenous molecule into a cell and testing for binding of the exogenous molecule to a binding site, a ZFP that binds to a target site, located within an accessible region, is designed. The designed ZFP can be introduced into the cell, or a nucleic acid encoding the designed ZFP can be designed and the designed nucleic acid can be introduced into the cell, where it will express the designed ZFP. Methods for the design and/or selection of ZFPs that bind specific sequences are disclosed in U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Methods for selection include, but are not limited to, phage display and in vivo selection.

In a preferred embodiment, an exogenous molecule is a zinc finger DNA-binding protein (ZFP). Certain ZFPs, their properties and their binding sequences are known in the art, as described supra. Furthermore, it is possible, for any particular nucleotide sequence, to design and/or select one or more ZFPs capable of binding to that sequence and to characterize the affinity and specificity of binding. See, for example, U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Certain sequences, such as those that are G-rich, are preferred as ZFP binding sites. Since a **three**-finger ZFP generally binds to a 9- or 10-nucleotide target site, in a preferred embodiment, an accessible region, present within a region of interest in cellular chromatin, is searched for one or more G-rich sequences of 9-10 nucleotides and, for each sequence so detected, a ZFP can be designed to bind those sequences. In addition, two three finger modules can be joined, via an appropriate linker domain, to form a six-finger protein capable of recognizing an 18-20 nucleotide target site. See, for example, PCT/US99/04441.

Plasmids were constructed to encode transcriptional effector proteins containing zinc finger domains designed to recognize target sites surrounding the transcriptional initiation site of the human vascular endothelial growth factor (VEGF) gene; i.e. within the +1 accessible region described in Example 7. The target site has the sequence 5'-GGGGAGGATCGCGGAGGCTT-3'(SEQ ID NO: 1), where the underlined T residue represents the major transcriptional startsite for the VEGF gene. A binding domain containing six zinc fingers, named VEGF 3a/1, was designed to bind to this 20-nucleotide target sequence. A **three-finger zinc finger domain**, VEGF 1 was designed to bind to the upstream 10-nucleotides of this target site having the sequence 5'-GGGGAGGATC-3' (SEQ ID NO: 2). A control six-fmger domain, GATA 15.5, which was designed to bind the sequence 5'-GAGTGTGTGAACTGCGGGGCAA-3' (SEQ ID NO: 3), was also used. These zinc finger domains were encoded as fusion proteins in the NVF vector, as described below.

> The zinc finger domains were constructed in a SP1 backbone. **The sequences of the recognition helices**, from position -1 to position +6, of VEGF 3a/1, VEGF 1 and GATA 15.5 are shown in Table 1. ...
>
> The zinc finger domains contained **designed recognition helices**, as shown in Table 1, in a SP1 backbone.
>
> An engineered fusion protein was designed to recognize a unique 9 base pair sequence in the DNase I hypersensitive region at –2 kb. This protein (BOS 3) comprised a nuclear localization sequence, a zinc finger binding domain, a KRAB repression domain and a FLAG epitope. The zinc finger binding domain was targeted to the sequence GGGGAGGAG, (SEQ ID NO: 27) which is complementary to the sequence CTCCTCCCC (SEQ ID NO: 28) in the coding strand. **Zinc finger sequences (for amino acids –1 through +6 of the recognition helices)** were RSDNLTR (SEQ ID NO: 29), RSDNLTR (SEQ ID NO: 30) and RSDALTK (SEQ ID NO: 31). Construction of a plasmid encoding the fusion protein and determination of the binding affinity of the zinc finger binding domain for its target sequence were performed according to methods disclosed in co-owned PCT WO 00/41566 and WO 00/42219. The dissociation constant (Kd) was determined to be 3.5 pM.

Indeed, the well-understood definition of "non-naturally occurring" was recently addressed by the Board of Patent Appeals and Interferences, where the Board confirmed that that even when the phrase "naturally occurring" does not appear verbatim in the specification, it would clearly be understood by the skilled artisan to mean something that exists or is found in nature. See, *Ex parte Dewis* (Appeal 2007-1610, decided September 4, 2007). Plainly, the skilled artisan would have no doubt as to the scope of the term "non-naturally occurring," namely to zinc finger proteins whose recognition helices are designed and/or selection and are not found in nature.

Thus, contrary to the Examiner's assertions, the evidence of record clearly establishes that, at the time of filing, the genus of non-naturally occurring zinc finger proteins was known (recognition helix modifications, backbone, cloning, functionality, etc.). The skilled artisan would know that a non-naturally occurring recognition helix (of

7 amino acids) could readily be inserted into any zinc finger backbone to bind to a selected target site.

Moreover, as evidenced by the number of references cited in the specification regarding design and/selection of zinc finger proteins, the state of the art at the time of filing clearly evidences that the skilled artisan would understand that a zinc finger proteins can be designed to bind to regions of cellular chromatin that are sensitive to digestion with DNAseI. The whole point of the entire specification is to describe binding of molecules (e.g., non-naturally occurring zinc finger proteins) to accessible regions of cellular chromatin. As clearly described and would be evident to the skilled artisan, one way accessible regions are identified is by digestions with DNAseI (page 4, lines 13-20 (paragraph [0015] of the published application); page 13, lines 20-30 (paragraph [0057] of the published application); page 15, lines 14-19 (paragraph [0063] of the published application); Examples 2, 5, 6 and 15; and the Figures, emphasis added):

> Accessible regions are determined, for example, by identifying regions in cellular chromatin that are hypersensitive to the action of various structural probes, either chemical or enzymatic. In a preferred embodiment, an enzymatic probe is used. In a more preferred embodiment, the enzymatic probe is deoxyribonuclease I (**DNase I**).
>
> In one embodiment, an enzymatic probe of chromatin structure is used to identify an accessible region. In a preferred embodiment, the enzymatic probe is **DNase I** (pancreatic deoxyribonuclease). Regions of cellular chromatin that exhibit enhanced sensitivity to digestion by DNase I, compared to bulk chromatin (i.e., DNase-hypersensitive sites) are more likely to have a structure that is favorable to the binding of an exogenous molecule, since the nucleosomal structure of bulk chromatin is generally less conducive to binding of an exogenous molecule. Furthermore, DNase-hypersensitive regions of chromatin often contain DNA sequences involved in the regulation of gene expression. Thus, binding of an exogenous molecule to a **DNase-hypersensitive** chromatin region is more likely to have an effect on gene regulation.
>
> In general, target sites for newly-discovered transcription factors, as well as other types of exogenous molecule, can be determined by methods that are well-known to those of skill in the art such as, for example, electrophoretic mobility shift assay, exonuclease protection, DNase footprinting, chemical footprinting and/or direct nucleotide sequence

> determination of a binding site. See, for example, Ausubel et al., supra, Chapter 12.

Thus, Applicants were also clearly in possession of complexes as claimed in which the zinc finger protein with the non-naturally occurring recognition helices is bound to a site in cellular chromatin that is sensitive to DNAse digestion.

Accordingly, for at least these reasons, Applicants submit that the no new matter has been added and withdrawal of the rejections is in order.

35 U.S.C. § 102

Claims 57, 68, 70 and 71 were rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 6,013,453 (hereinafter "Choo"), which was alleged to disclose the making of a mutant 3-fingered zinc finger protein that binds to the "coding sequence for a specific ras mutation" in human cells. (Final Office Action, page 16). It was also alleged that "absent reason to believe otherwise, this site occurs within the broad definition of a general region which is in some way sensitive to digestion with DNAseI." *Id.*

Applicants traverse the rejection and supporting remarks.

The Examiner errs in asserting that Choo teaches that their zinc finger protein binds to a site in cellular chromatin, let alone that the in vitro sequence bound by their protein is necessarily an accessible region in cellular chromatin. Rather, Example 5 (not Example 4 as cited in the Office Action), teaches that experiments in cells have yet to be done and, moreover that the planned prophetic experiments proposed by Choo do not involve cellular chromatin, but, rather plasmids (Example 5 of Choo, emphasis added):

> Following selection, a number of separate clones were isolated and phage produced from these were screened by ELISA for binding to the G12V ras sequence and discrimination against the wild-type ras sequence. A number of clones were able to do this, and sequencing of phage DNA later revealed that these fell into two categories, one of which had the amino acid Asn at the +3 randomised position, and another which had two other undesirable mutations.

> Assay of the protein in eukaryotes (e.g. to drive CAT reporter production) requires the use of a weak promoter. When expression of the anti-RAS (G12V) protein is strong, the peptide presumably binds to the wild-type ras allele (which is required) leading to cell death. For this reason, a regulatable promoter (e.g. for tetracycline) will be used to deliver the protein in therapeutic applications, so that the intracellular concentration of the protein exceeds the Kd for the G12V point mutated gene but not the Kd for the wild-type allele. Since the G12V mutation is a naturally occurring genomic mutation (not only a cDNA mutation as was the p190 bcr-abl) human cell lines and other animal models can be used in research.

This is not a demonstration about engineered zinc finger proteins forming complexes with cellular chromatin. Indeed, evidence of record establishes that the skilled artisan had absolutely no expectation cellular chromatin could be complexed with an engineered zinc finger protein. The cited reference, Choo, clearly teaches it was **not** predictable that engineered zinc finger proteins would bind to cellular chromatin: (see, Choo, col. 28, lines 39-47, emphasis added):

> The selective stimulation of transcription indicates convincingly that highly site-specific DNA-binding can occur in vivo. However, while transient transfections assay binding plasmid DNA, the true target site for this and most other DNA-binding proteins is genomic DNA. This might well present significant problems, not least since this DNA is physically separated from the cytosol by the nuclear membrane, but also since it may be packaged within chromatin.

Choo clearly states that endogenous cellular genes are different from artificial substrates, not only due to the presence of the nuclear membrane but due to the "normal chromatin environment." In addition, Choo clearly states that their randomly integrated sequences (or their episomal reporter plasmids) are not in their normal chromatin environment.

Therefore, the evidence clearly establishes that Choo does not describe or demonstrate complexes as claimed – this reference does not describe complexes of engineered zinc finger proteins and a region of cellular chromatin that is sensitive to digestions with DNAse. As such, Choo cannot anticipate any of the pending claims

35 U.S.C. § 103

Claims 57 and 68-71 were also rejected under 35 U.S.C. § 103(a) as allegedly obvious over Choo in view of WO 00/9837755 (hereinafter "Dangl"). (Final Office Action, pages 16-17). Choo was cited as above and Dangl was cited for teaching that zinc finger proteins function in plant cells. *Id.*

For the reasons detailed above, Choo does not describe or demonstrate the claimed complexes. To the contrary, Choo teaches that complexes of zinc finger proteins with non-naturally occurring recognition helices and cellular chromatin was entirely unpredictable. Thus, the claimed complexes are not a predictable use of the individual elements, and, as such, these claims cannot be obvious over any combination of Choo and Dangl.

Still further evidence that Choo's disclosure was not seen by the skilled artisan as providing any reasonable expectation that engineered zinc finger proteins would form complexes with DNAse-sensitive regions of cellular chromatin is also of record. Well after Choo's work, the laboratories working in the field of zinc finger proteins clearly indicated that their skilled artisans regarded modulating endogenous gene expression with an engineered zinc finger protein as an unmet challenge:

> While our early experiments have focused on the regulation of genes transiently introduced into cells, we realized that the willful and specific regulation of endogenous genes with designed transcription factors has remained an unmet challenge in biology.[1]

In a separate discussion article, a co-author of this Beerli paper also discussed the complete lack of predictability of using ZFPs to bind to endogenous genes based on studies such as Choo's (which used introduced binding sites):

[1] See, Beerli et al. (2000) Proc. Natl. Acad. Sci. USA 97:1495-1500, page 1465, left column, reference AH-1 of IDS submitted May 3, 2002 and May 7, 2004 and indicated considered by the Office July 19, 2004.

> "This is the first time we've been able to show that these designed transcription factors work on real genes and real chromosomes, not genes of binding sites that have been introduced into cells,"....[2]

Thus, the skilled artisan is on the record as stating that binding of engineered zinc finger proteins to cellular chromatin was unexpected based on Choo's disclosure.

Given the clear teaching away from binding engineered zinc finger to cellular chromatin by Choo, there is no combination of Choo and Dangl that render any of the pending claims obvious over these references. Therefore, withdrawal of the rejection is in order.

[2] See, Borman "DNA-Binding Proteins Turn Genes On and Off." February 21, 2000, C&CEN (copy attached hereto).

CONCLUSION

For the reasons set forth herein, allowance of the claims under consideration, and rejoinder and allowance of the withdrawn claims, are requested.

Respectfully submitted,

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DNA-Binding Proteins Turn Genes On And Off

Scientists at Scripps Research Institute, La Jolla, Calif., have designed proteins that bind DNA in a highly specific manner and act like transcription factors to turn endogenous genes on and off in living cells. Transcription factors are DNA-binding proteins that activate the transcription of DNA into messenger RNA.

Postdoctoral fellows Roger R. Beerli and Birgit Dreier and molecular biology professor Carlos F. Barbas III report the findings in the *Proceedings of the National Academy of Sciences USA* [97, 1495 (2000)].

"This is the first time we've been able to show that these designed transcription factors work on real genes and real chromosomes, not genes or binding sites that have been introduced into cells," Barbas says. "We can really knock out expression in a controlled way or elevate the level of expression from the native level to far beyond it to study the role of proteins within the cell." He notes that the ability of the designed transcription factors to bind 18-base-pair sequences makes it possible for each of them to target a unique site within the human genome.

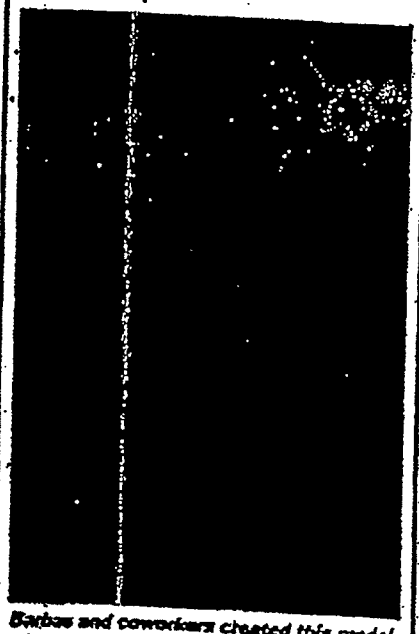
Barbas says that his group's technology can potentially be used to regulate genes of pharmaceutical interest, attack cancer or genetic diseases, control crops, and even "to make flowers bloom when you want them to." But he believes the technology also has great potential as a basic research tool. "One could make switches that turn on and turn off genes in a very high-throughput fashion and study the phenotypes of the cells that result," he says.

Chemistry professor Peter B. Dervan of California Institute of Technology calls the study by Barbas and coworkers "a step forward. Long term, designed transcription factor mimics are a whole new approach to human therapeutics," he says.

"There are several milestones one needs to overcome in the design and discovery of artificial transcription factors to control gene expression, and hence human disease," Dervan adds. For example, "putting artificial proteins in human cells will require gene therapy techniques. But that is a medical technology that will likely be successful in our lifetime."

The synthetic transcription factors developed by Barbas and coworkers are designed proteins containing DNA-binding elements called zinc fingers, structures found in some natural transcription factors. Zinc fingers—independently folding domains of about 30 amino acid residues centered on a zinc ion—were discovered in 1985 by molecular biologist Aaron Klug and coworkers at the Medical Research Council Laboratory of Molecular Biology, Cambridge, England. "We called them zinc fingers because they grip or grasp DNA," Klug says.

Natural transcription factors often have both a DNA-binding domain that localizes the protein to a specific site and one or more effector domains that activate or repress transcription at or near that site. In the designed transcription factors devised by Barbas' group, synthetic zinc finger DNA-binding domains, which are designed to target specific sequences, are combined with naturally derived effector domains.



Barbas and coworkers created this model of a designed transcription factor with six zinc finger units (blue) bound to a DNA double helix (orange and red). They have designed transcription factors that can recognize a specific DNA sequence and repress or activate the gene containing that sequence.

In their *PNAS* paper, Barbas and coworkers show that these designed zinc finger proteins can repress or activate expression of the endogenous genes *erbB-2* and *erbB-3* in living mouse, monkey, and human cells. *ErbB-2* is frequently overexpressed in human breast and ovarian cancers, and expression of *erbB-3* also is suspected to be associated with cancer. In *erbB-2* and *erbB-3*, the 18-base-pair sites targeted by the synthetic proteins share 15 base pairs of common sequence, yet the transcription factors are specific enough to regulate one and not the other. The researchers also demonstrate that their transcription factors, and hence gene expression, can be regulated with the drug tetracycline.

Staff scientist Roy Pollock of Ariad Gene Therapeutics, Cambridge, Mass., who specializes in eukaryotic transcriptional regulation, comments that the sequence specificity of the transcription factors in the study is very impressive. He believes the Barbas study is the first to demonstrate both inhibition and activation of endogenous genes by designed transcription factors, rather than inhibition alone.

The findings suggest the possible use of designed transcription factors for gene therapy of cancer and other conditions. "Novartis has taken a license to our technology for the control of genes in plants," Barbas says, and Sangamo BioSciences, Richmond, Calif., "holds a license for the use of these proteins in human therapy."

Sangamo's president and chief executive officer, Edward O. Lanphier, points out that in unpublished studies the company has demonstrated the ability of engineered zinc finger DNA-binding proteins to control a number of endogenous genes. Sangamo has collaborative agreements with several other companies, including one with Baxter Healthcare to develop and commercialize zinc finger DNA-binding proteins that activate vascular endothelial growth factors (VEGFs) and VEGF receptors for treatment of vascular and cardiovascular diseases.

Several other research groups have developed or are developing related strategies for controlling gene expression. About six years ago, Klug and coworkers, including staff scientist Yen Choo, designed a zinc finger protein that bound specific DNA sequences in a leukemia gene and stopped uncontrolled cell

growth in live mouse cells [*Nature*, 372, 642 (1994)]. This was the first report of a DNA-binding protein engineered de novo to inhibit gene expression.

The group of Carl O. Pabo, professor of biophysics and structural biology and a Howard Hughes Medical Institute investigator at Massachusetts Institute of Technology, has developed phage-display methods to identify zinc finger proteins that recognize specific sequences. Pabo and coworkers have also fused zinc finger motifs to other DNA-binding elements to create hybrid transcription factors with novel sequence specificities—a technology to which Ariad Gene Therapeutics has obtained an exclusive license. One such hybrid has been used successfully by Ariad researchers to bind to and activate synthetic therapeutic genes introduced into animals.

Ariad also is developing a technique in which a DNA-binding domain and an activation domain come together, in the presence of an orally available drug, to form a transcription factor. The idea is to eventually be able to use such transcription factors to turn synthetic genes on and off in patients for gene therapy applications.

And Dervan and coworkers have developed cell-permeable polyamides that fold into hairpin loops and undergo sequence-specific binding to base pairs in the minor groove of DNA, where they can play a regulatory role. Binding of these polyamides modulates gene expression by blocking other proteins, such as transcription factors, that would otherwise be able to bind to those sites. The researchers demonstrated three years ago that polyamides targeted at key sequences in the promoter region of a human immunodeficiency virus gene strongly inhibited viral replication [*Proc. Nat. Acad. Sci. USA*, 95, 12890 (1998)].

In unpublished work, Dervan's group and that of professor Mark S. Ptashne, head of the Gene Regulation Laboratory at Sloan-Kettering Institute, New York City, have created artificial transcription factors in which a small peptide activation domain is combined with a DNA-binding polyamide. The collaborative team finds that these synthetic transcription factors are capable of activating gene transcription in vitro. Dervan says he is particularly excited about these findings since previous polyamide constructs have been able to repress gene expression but not to activate it.

Dervan's synthetic polyamides can zero in on most any DNA sequence. The Barbas group's designed zinc finger proteins can't do that yet. They bind up to 18-unit chunks of repeating GNN triplet sequences, where G is guanine and N is any of the other three types of DNA bases. Barbas and coworkers have reported the development of 16 distinct types of zinc finger domains, but 64 such domains would be required to bind any sequence at will.

Nevertheless, Barbas says, "it turns out that stretches of sequence that repeat the GNN motif six times are actually quite common in genes, and in every gene we've looked at we can find at least one of those sites. And we don't have to place the protein at very specific sites within the gene to regulate it. So we should be able to control most genes with our 16 zinc finger domains, and we believe that many of the 64 codon-recognition domains will become accessible in the next year or two." Barbas notes that in unpublished studies his group has prepared many of the additional domains required for the recognition of any given sequence.

Choo, Klug, and coworkers have already developed a new zinc finger display strategy that makes it possible to target more or less any 18-base-pair DNA sequence—not just repeating GNN triplets. A scientific paper on the new strategy "is already written up and ready to go off," Choo notes, but some of the information has already appeared in patent applications filed by Gendaq Ltd., London, a company founded last year by Choo and Klug. At Gendaq, "we aim to apply customized transcription factors for gene regulation in functional genomics, agricultural biotechnology, and human therapy," Choo says.

With regard to potential applications of designed transcription factors, Barbas points out that gene therapy trials have been carried out in which patients with heart ailments have been injected with viruses containing VEGF genes. An alternative approach, he says, "would be simply to activate the endogenous VEGF gene found in every cell with a designed transcription factor." Furthermore, "you could imagine introducing a zinc finger protein that activates insulin and is under the control of a nontoxic, orally available pill," such as an aspirin-like derivative.

"We have encoded in our own genes the solutions to many diseases," Barbas says. "What awaits is just a way to switch on those critical genes."

Stu Borman

Enzyme's Activity Designed To Order

Researchers in England have modified a natural enzyme into a designed enzyme with an entirely new catalytic function. They accomplished this by cutting two sections out of the gene for the natural enzyme, inserting replacements containing mutations, shuffling the mutations, and screening expressed libraries of modified proteins for the desired activity—a process called directed evolution [*Nature*, 403, 617 (2000)].

Alan R. Fersht and coworkers at Cambridge Centre for Protein Engineering and Cambridge University Chemical Laboratory created the designed enzyme by substituting modified catalytic or binding units into an enzyme with an α/β-barrel structure—a common type of protein structural foundation or "scaffold."

In the study, Fersht and coworkers aimed to convert the activity of indole-3-glycerol phosphate synthase (IGPS) into that of phosphoribosylanthranilate isomerase (PRAI). The deck was loaded somewhat in that the two enzymes catalyze sequential in vivo reactions in the tryptophan biosynthetic pathway and PRAI's catalytic product is IGPS's substrate. Nevertheless, the activities of the two enzymes are quite different, so it was no easy feat to get IGPS to mimic PRAI's catalytic function.

To do so, Fersht and coworkers snipped substrate-binding and catalytic loops out of the IGPS gene, inserted modified sequences in the gaps, shuffled the mutations, and screened the expressed enzyme variants for PRAI-like activity by testing them in PRAI-deficient bacteria that need tryptophan to grow. In the end, the researchers identified a modified IGPS with an activity and catalytic efficiency strikingly similar to those of PRAI.

The work has potential applicability to the creation of novel biocatalysts, and it advances scientists' understanding of the type of natural evolutionary mechanisms that enzymes might use to develop new functions and adapt to changing environments. The findings also suggest that the α/β-barrel scaffold—which may be found in as many as 10% of all soluble enzymes—could be generally useful for creating biocatalysts with a range of novel activities.

"Alan was trying to demonstrate that